$640.00 petition fee as set forth in 37 C.F.R. § 1.17(m) for a small entity in compliance with 37 C.F.R. § 1.27(a).

TECHNICAL AMENDMENTS:

IN THE TITLE:

Please amend the title of the application to read:

B1

--MELANOMA DIFFERENTIATION ASSOCIATED GENE-5 AND VECTORS AND CELLS CONTAINING SAME--

IN THE SPECIFICATION:

Please amend the paragraph beginning on page 4, line 3, of the specification to read:

B2

--Figures 1A-1D. Sequence of mda-5 and alignment with CARD and RNA helicases. Figure 1A. Nucleotide sequence (SEQ ID NO:1) and corresponding amino acid sequence (SEQ ID NO:2) of mda-5. Underlined sequences are AUUUA sequences. Bold face sequence is the poly A signal. Figure 1B. Additional nucleotide sequence of mda-5p (SEQ ID NO:4). Poly A signal is bold faced. Figure 1C. Alignment of CARD proteins with 50 amino acids near the N-terminal region of MDA-5 (a.a. 125-174 correspond to 1-50). (SEQ ID NOS:5-11) Figure 1D. Alignment of the RNA helicase conserved motif of mda-5 with eIF-4A (SEQ ID NO:12) and p68 RNA helicases-2E (SEQ ID NO:13).--

Please amend the paragraph beginning on page 4, line 33, of the specification to read:

B3

--Figures 3A-3B. Northern blot analysis of mda-5 expression induced by IFN-β in

B3 **normal and tumor cell lines.** RNA samples were extracted from the indicated cells treated with 2,000 U/ml of interferon-β for 24 hr. Northern hybridization was performed as in Materials and Methods. cont.

Please amend the paragraph beginning on p. 17, line 12, of the specification to read:

B4 --In one embodiment of the invention, the promoter comprises the nucleotide sequence shown in SEQ ID NO:3.—

Please amend the paragraph beginning on page 57, line 22, of the specification to read:

--Electronic sequence analysis of the MDA-5 protein using motif and profile scans of proteins presently in the protein database identified two conserved domains, a caspase recruitment domain (CARD) and an RNA helicase domain. The CARD domain which was defined by generalized profile alignment within the RAIDD and ICH-1 amino terminal regions, is present in various apoptotic molecules such as Mch6, ICE, ICH-2, c-IAP1, c-IAP2 and Ced-3. Current evidence suggests that the biological role of CARD is the recruitment of caspase to apoptotic signaling receptor complexes (19). The sequence alignment of N-terminal 50 amino acids (aa 125-174) of MDA-5 with other CARD-proteins reveals significant sequence homology at conserved amino acids of CARD (Figure 1B). MDA-5 displays the highest homology to the CARD region of RAIDD, which is involved in TNF-R1-mediated apoptotic signal transduction (Figure 1C) (19). The C-terminal 100 amino acids (aa 722-823) of MDA-5 also show significant sequence homology to the RNA helicase C-terminal conserved domain, which is involved in RNA binding and unwinding of double-stranded RNA (Figure 1D) (20). In addition, as with other RNA helicases MDA-5 also contains an ATPase A and B motif (331-TGSGKT; SEQ ID NO:14 and 443-DECH; SEQ ID NO:15)

B5

(Figure 1D) (20). However, MDA-5 has unique features in its helicase C-terminal motif and ATPase A motif. MDA-5 has ARGRA (SEQ ID NO:16) instead of the well-conserved YIHRIGRXXR (SEQ ID NO:17) motif, which is critical for RNA binding in other RNA helicases (20). The ATPase A motif of MDA-5 (LPTGSGKT; SEQ ID NO:18) is also different from the consensus sequence motif (A/GXXGXGKT; SEQ ID NO:19) found in other RNA helicases (20). Moreover, MDA-5 is the first putative RNA helicase that retains both an altered RNA binding motif and an ATPase A motif. Screening of the SwissProt database for homologous sequences containing both of these motifs identified three yeast hypothetical ORFs encoding putative helicases (Gen Bank Accession Number Q09884, Q58900 and P34529). The unique features conserved in *MDA-5* and these yeast proteins may signify that MDA-5 is a member of a new family of helicases. RNA helicases are known to be involved in diverse cellular processes including RNA splicing, RNA editing, RNA nuclear cytosolic transport, translation and viral replication by ATP-dependent unwinding of dsRNA (20). However, based on the unique structure of MDA-5, it is not possible at present to ascribe a biological role for this new molecule and new family of helicases.—

Please amend the paragraph beginning on page 71, line 24 of the specification to read:

--Another distinct motif present in the MDA-5 protein is a RNA helicase signature domain, which spans the C-terminal half of this molecule. RNA helicase is a family of enzymes with a helicase motif, which potentially catalyzes NTP-dependent dsRNA unwinding activity. Not only are the core residues among the RNA helicases conserved, but also the spaces between these residues are retained in the different RNA helicases. Three main features characterize RNA helicases from the N- to C-terminal, an ATPase A motif (GXXGXGKT), an ATPase B motif (DEAD; SEQ ID NO:20, DEAH; SEQ ID NO:21 or DEXH; SEQ ID NO:22) and a critical domain for RNA

interaction (HRIGRXXR; SEQ ID NO:23). RNA helicases are classified into three subgroups based on their ATPase B motifs. RNA helicases are implicated in the majority of steps associated with RNA processing and transcription, nuclear and mitochondrial RNA splicing, RNA editing, ribosomal biogenesis, nuclear cytosolic RNA export, degradation of nonsense RNA and RNA translation. Hence, RNA helicases affect many biological phenomena including cell differentiation, proliferation, development and viral life cycle. Although the RNA helicases are classified into three subgroups, the biological relevance of these groups remains to be defined. In addition, the enzymatic activity of many putative RNA helicases has not been confirmed, this could partly be because of the absence of the appropriate substrate and standard protocol due to the diversity of these enzymes.—

B6 Cont.

Please amend the paragraph beginning on page 72, line 13 of the specification to read:

--Despite the well-conserved attributes of RNA helicases, MDA-5 contains four unique features that could mediate functional divergence. The CARD domain of MDA-5 in its N-terminal region is not found in any previously identified helicases, although the functional significance of this region is currently under investigation. The ATPase A motif of mda-5 is unique and contains LPTGSGKT as opposed to the sequences found in other RNA helicases (GXXGXGKT) and a mutation of the first glycine residue of murine eIF-4A to valine abolishes ATP binding ability. Since leucine is a non-polar amino acid as is valine, but it has a bulkier side chain than valine, MDA-5 may not bind ATP effectively and, hence, may be an ATPase defective helicase or it may require a different energy source and/or metals for activity. This property of MDA-5 may explain the reduction in colony forming efficiency by a expression of a mutant of mda-5 lacking this region of the MDA-5 protein. The HRIGRXXR motif which is critical for RNA binding in vitro is not well conserved in MDA-5 (ARGRI; SEQ ID NO:24). The functional role of such sequence divergence in the MDA-5 protein

B7

B7 Cont.

remains to be determined. Three yeast hypothetical ORFs share specific features of MDA-5 including ATPase and RNA binding sites, but their biological function has not been ascertained. Complementation assays between these proteins can provide insights on functional and evolutionary relationship among these molecules.--

Please amend the paragraph beginning on page 84, line 1, of the specification to read:

--Abstract of the Disclosure

B8

The invention provides for isolated nucleic acids encoding an Mda-5 polypeptide as shown in SEQ ID NO:1. The invention also provides for isolated nucleic acids comprising derivatives of the sequence of SEQ ID NO:1 that encode polypeptides functionally equivalent to Mda-5. The invention further provides for fragments of the isolated nucleic acid of SEQ ID NO:1 that encode polypeptides having Mda-5 biological activity. Vectors comprising these isolated nucleic acids and host cells comprising these vectors are also provided by the instant invention.—

Please replace the paper copy of the Sequence Listing previously filed with the paper copy of the Sequence Listing enclosed herewith, inserting the Sequence Listing into the specification after p. 84. The Sequence Listing thus becomes pages 85-98 of the specification.

IN THE DRAWINGS:

Please substitute the corrected version of Figure 9 contained herein, wherein the corrections are marked in red on the attached version of the marked-up copy of this figure, for the original Figure 9.